Optimization of Reducing Sugar Production from Acid Hydrolysis of Taro Root Powder by Response Surface Methodology

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Abstract

In this research work, taro roots were used as a source for preparation of reducing sugar by acid hydrolysis. The influencing factors of acid hydrolysis using sulphuric acid such as acid volume, acid concentration and hydrolyzing time on the yield of reducing sugar were investigated. During acid hydrolysis, Box-Behnken design of response surface methodology (RSM) was used to optimize the influencing variables. The estimated values for acid volume, acid concentration and hydrolyzing time were 90.55 mL, 7.42% v/v and 94 min for acid hydrolysis of taro roots for reducing sugar, 553 mg/g.

Keywords : taro roots, sulphuric acid, acid hydrolysis, reducing sugar, Box-Behnken design

Introduction

Taro is a common name for the corms and tubers of several plants in the family Araceae. Its botanical name is *Colocasia esculenta.* The fresh corms contain 60-80 % water, 13-30 % carbohydrate, mostly small and very digested starch grains (the majority being starch of which 17-28% is amylase, and the remainder is amylopectin), 1.5-3 % protein and vitamin C, thiamine, riboflavin and niacin (Cobley,1976).

Starch consists of long chains of glucose molecules and can also be converted to fermentable sugar by hydrolysis. Hydrolysis is a reaction of

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starch with water, which is normally used to break down the starch into fermentable sugar. There are two types of hydrolysis; enzymatic hydrolysis and acid hydrolysis (Balat et al., 2008).

Acid Hydrolysis of starch is relatively simple, but it requires acid proof equipment, high temperatures, and the handling of acid. Basically, dilute mineral acid (usually sulfuric acid) is added to the grain slurry prior to cooking at a concentration of 1-4% as calculated on a weight/weight basis.The mash is then cooked. Cooking and conversion of the starch take place simultaneously. The mash is then immediately neutralized with calcium hydroxide (lime), or some other base, and fermented as usual. This process is unsuitable for small scale use. However, it is an excellent process for large operations because cooking time is short and the method is readily adaptable to continuous operation. However, acid hydrolysis is extremely hazardous, required corrosion-resistant vessels and resulted in a product with a very high salt content, due to neutralization. In addition, dextrose yield was limited to approximately 85(% w/w) and off-colors and flavors were often a result of the acid hydrolysis process (Kearsly, 1995).

Materials and Methods

Materials

Fresh taro roots in medium size were purchased from Taunggyi District, Shan State. Caustic soda (BDH, analar grade), hydrochloric acid (sp gr = 1.18) and sulphuric acid (sp gr = 1.84) (BDH, analar grade) were purchased from Kemiko Chemical dealer, Pabeden Township, Yangon Region.

Figure 1. Taro Roots

Methods

Preparation of Taro Root Powder

Fresh taro roots were washed with running water and allowed to dry at room temperature for about 1 hour. The outer skin was peeled off and sliced into small pieces which were then dried at 60°C in a hot air oven for about 5 hours. The dried chips were then ground into fine powder and sieved through an 80 mesh stainless steel screen. The samples were stored in the airtight bottles.

Chemical Analysis of Taro Stems, Taro Roots and Cassava Roots

Determination of moisture content, ash content and starch contents were conducted at the laboratory of Department of Industrial Chemistry, University of Yangon. Contents of protein, crude fiber and crude fat were determined at Food Industries Development Supporting Laboratory (FIDSL), UMFCCI Tower, Lanmadaw Township, Yagon Region.

Preparation of Reducing Sugar from Taro Root Powder by Acid Hydrolysis

Taro root powder, 25 g was mixed with 100 ml of distilled water and heated at 70°C for 30 min to gelatinize the slurry. After gelatinization, the viscous solution was mixed and stirred with 90.55 ml of 7.42 % (v/v) sulphuric acid in a conical flask. Then, the slurry was boiled for 94 minutes. After boiling, the warm hydrolysate was directly neutralized with 2 M caustic soda solution and filtered through filter cloth.

Optimization of Acid Hydrolysis of Taro Root Powder for the Yield of Reducing Sugar

Box-Behnken Design was also used for experimental design of response surface methodology (RSM). Acid concentration, acid volume and hydrolyzing time were the influencing factors for the response of reducing sugar and the experimental runs are tabulated in Table (2). The limits are shown in Table (1).

Table 1. Variables in Experimental Design for Acid Hydrolysis of Taro Root Powder

Sr No.	Variables	Levels		
		Lower -1	Basis 0	Upper +1
1	Acid volume (mL) , x_1	50	100	150
2	Acid concentration (% v/v) , x_2	5	7	9
3	Hydrolysis Time (min), x_3	30	60	90

Table 2. Experimental Runs for Acid Hydrolysis of Taro Root Powder according to Box-Behnken Design

Run No.	Coded			Uncoded		
	x_1	x_2	x_3	X_1	X_2	X_3
1	-1	-1	0	50	5	60
2	-1	1	0	50	9	60
3	1	-1	0	150	5	60
4	1	1	0	150	9	60
5	-1	0	-1	50	7	30
6	-1	0	1	50	7	90
7	1	0	-1	150	7	30
8	1	0	1	150	7	90
9	0	-1	-1	100	5	30
10	0	-1	1	100	5	90
11	0	1	-1	100	9	30
12	0	1	1	100	9	90
13	0	0	0	100	7	60
14	0	0	0	100	7	60
15	0	0	0	100	7	60

Determination of Reducing Sugar by Lane and Eynon Method

Fehling's solution, 10 mL was added into a 250 mL conical flask and 10 mL of sugar solution was added into the Fehling solution. The mixture was then boiled and 1mL of sugar solution was added at a time during boiling until the color of the solution changed from blue to red. A few drops of methylene blue indicator were added and titration was continued until the color turned red (Pearson, 1976). The reducing sugar content was then calculated.

$$\text{Reducing Sugar, (\% w/w)} = \frac{\text{Factor x Y x 100}}{\text{Titre x W}}$$

Where,

Y = volume of sugar solution

W = weight of sample

Results and Discussions

In this research work, reducing sugar was prepared from taro root powder. Table 3 shows the chemical composition of taro root power. Taro root powder has 79.26 (% w/w), starch content. The starch content related to the yield of sugars, namely, glucose because of its formulation of large glucose units joined together by glucosidic bonds((Fisher, 2004).

Table 3. Chemical Compositions of Taro Root Powder

Sr No.	Components	Content (% w/w)
1	Moisture	10.04
2	Ash	3.25
3	Protein	5.95
4	Crude Fiber	0.99
5	Crude Fat	0.51
6	Starch	79.26

For preparing reducing sugar from taro roots, firstly, taro root powder was gelatinized with distilled water at 70°C for 30 min to break down the intermolecular bonds of starch molecule and to improve the availability of starch for hydrolysis. After gelatinization, both acid hydrolysis can be conducted for conversion into fermentable sugar.

Acid hydrolysis of taro root powder was also conducted by using sulphuric acid according to the experimental runs in Box-Behnken design of response surface methodology (RSM). The second order polynomial equation obtained by using the statistical software (Design Expert, version 7, Stat Ease Inc.,) for the response function of reducing sugar is shown in Equation (1).

$$\begin{aligned}\text{Fermentable Sugar, mg/g} = {} & 553.50 + 36.61375x_1 + 58.66x_2 + 22.42x_3 \\ & +19.595x_1x_2 - 2.4875x_1x_3 - 38.7175x_2x_3 \\ & -82.525x_1^2 - 122.33x_2^2 - 7.2325\,x_3^2 \quad \text{----Eq}^{\underline{n}}\ (1)\end{aligned}$$

The adequacy of the developed quadratic model was also justified through analysis of variance (ANOVA).The results of ANOVA analysis for regression equation are presented in Table 4..The present data of correlation coefficient R^2 also indicated the high degree correlation between the observed values and the predicted values of fermentable sugar. Moreover, adjusted R^2 was also very close to correlation coefficient R^2 meaning that significant terms have been included in the fitting model. The F value of 7.2 implied that the model was significant (Montgomery, 2005). By solving the equation (1) by using MATLAB, the estimated value of fermentable sugar was 553 mg/g by using 90.55 mL of 7.42 (% v/v) at hydrolyzing time of 94 min. The interactive effects between acid volume and acid concentration, acid volume and hydrolyzing time, acid concentration and hydrolyzing time on the yields of fermentable sugar were presented as 3 dimensional response surface plots and contour plots as shown in Figures (2), (3), and (4) respectively.

Table 4. Summary Output of Analysis of Variance (ANOVA) for Response Surface Quadratic Model for Acid Hydrolysis of Taro Root Power

Sr No.	Regression Statistics	Outputs
1	R-Squared	0.9009
2	Adjusted R-Squared	0.7738
3	Predicted R-Squared	-0.5853
4	Adequate Precision	7.972
5	Std. Deviation	48.14

Table 5. Amount of Observed and Predicted Fermentable Sugar for acid Hydrolysis of Taro Root Powder

Run No.	x_1	x_2	x_3	Amount of Observed Reducing Sugar (mg/g)	Amount of Predicted Reducing Sugar (mg/g)
1	50	5	60	323.26	272.96
2	50	9	60	357.19	351.09
3	150	5	60	300.96	307.00
4	150	9	60	413.22	463.51
5	50	7	30	401.39	402.22
6	50	7	90	396.53	452.03
7	150	7	30	535.93	480.42
8	150	7	90	521.12	520.28
9	100	5	30	254.66	304.14
10	100	5	90	431.64	426.41
11	100	9	30	493.67	489.89
12	100	9	90	513.78	466.5
13	100	7	60	535.50	553.5
14	100	7	60	535.45	553.5
15	100	7	60	535.52	535.52

The experiments were conducted at the Laboratory of Industrial Chemistry Department, Dagon University.

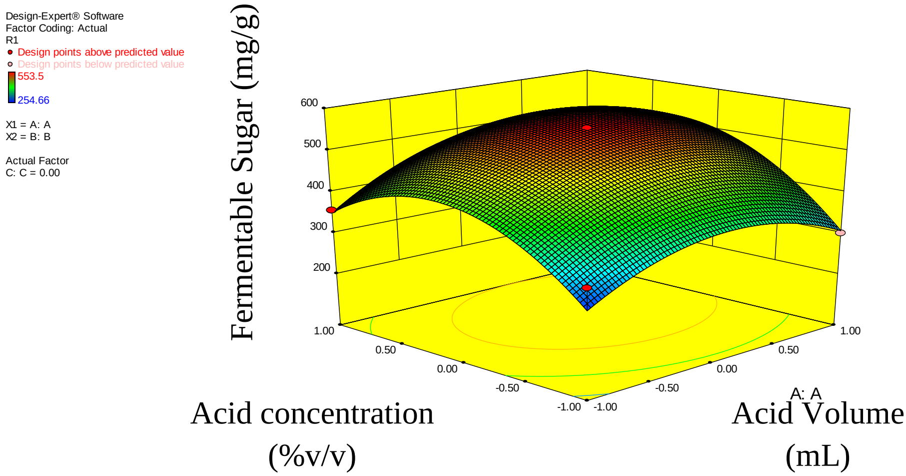

Figure 2. 3D Response Surface Plot for the Maximum Fermentable Sugar by RSM as Function of the Acid Volume and the Acid Concentration at the Hydrolyzing Time of 94 min in Acid Hydrolysis of Taro Root Powder

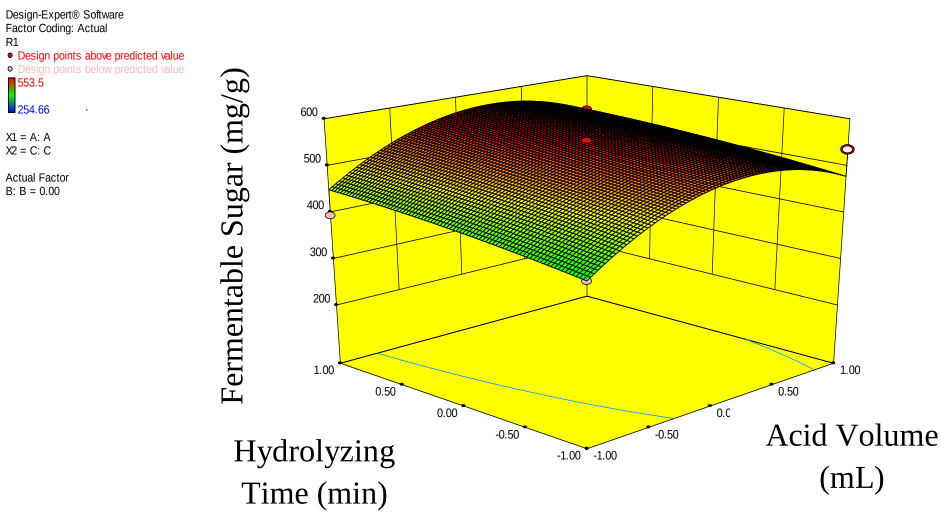

Figure 3. 3D Response Surface Plot for the Maximum Fermentable Sugar by RSM as Function of the Acid Volume and the Hydrolyzing Time at the Acid Concentration of 7.42 (% v/v) in Acid Hydrolysis of Taro Root Powder

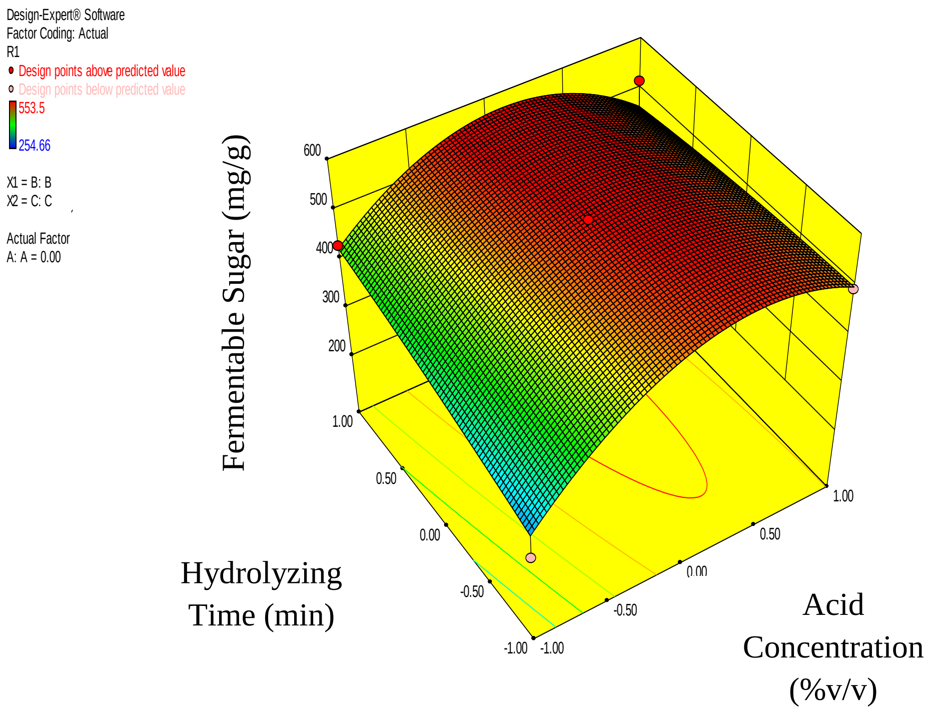

Figure 4. 3D Response Surface Plot for the Maximum Fermentable Sugar by RSM as Function of the Acid Concentration and the Hydrolyzing time at the Acid Volume of 90.55 mL in Acid Hydrolysis of Taro Root Powder.

Conclusion

In this study, reducing sugar was prepared from taro root powder by acid hydrolysis using sulpuric acid. The maximum yields of reducing sugar 553 mg/g have been achieved under the condition optimized by response surface methodology (RSM).

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